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FILE 'HCAPLUS' ENTERED AT 11:46:40 ON 04 AUG 2004
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FILE COVERS 1907 - 4 Aug 2004  VOL 141 ISS 6
FILE LAST UPDATED: 3 Aug 2004  (20040803/ED)

 This file contains CAS Registry Numbers for easy and accurate
 substance identification.

=> d his

     (FILE 'HOME' ENTERED AT 11:09:30 ON 04 AUG 2004)
               SET COST OFF

     FILE 'HCAPLUS' ENTERED AT 11:09:46 ON 04 AUG 2004
L1             1 S US20040049012/PN OR (WO2003-US28007 OR US2002-408653#)/AP,PRN
               E GEHANT R/AU
L2             8 S E6-E8
L3             2 S L2 AND (BIOCHEM?(L)METHOD?)/SC,SX
L4             1 S L3 AND PROTEIN EXTRACTION
L5             1 S L1,L4
               E E COLI/CT
               E ESCHERICHIA COLI/CT
L6        137058 S E3
               E E3+ALL
L7        137078 S E6,E5+NT
L8        239078 S ("E" OR ESCHER?)()COLI
L9        239090 S L6-L8
L10          290 S L9 AND EXTRACTION/CW
               E EXTRACTION/CT
               E E3+ALL
L11          370 S L9 AND E2+NT
L12        17966 S L9 AND EXTRACT?
L13        18034 S L10-L12
L14         8737 S L13 AND ?PROTEIN?
L15         4835 S L13 AND PROTEIN?/CW
L16         8737 S L14,L15
               E CHROMATOGRAPHY/CT
L17          267 S L16 AND E3+OLD,NT,PFT,RT
L18           72 S L16 AND E27+OLD,NT,PFT,RT
L19           75 S L16 AND E28+OLD,NT,PFT,RT
L20           68 S L16 AND E31+OLD,NT,PFT,RT
L21            3 S L16 AND E111+OLD,NT,PFT,RT
L22           13 S L16 AND E146+OLD,NT,PFT,RT
L23            0 S L16 AND E152+OLD,NT,PFT,RT
L24            0 S L16 AND E155+OLD,NT,PFT,RT
L25            2 S L16 AND E159+OLD,NT,PFT,RT
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- 2 upper bound

103 reverse steps

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                E E3+ALL
L26             0 S L16 AND E6
L27            82 S L16 AND E384+OLD,NT,PFT,RT
L28            87 S L16 AND E385+OLD,NT,PFT,RT
L29           219 S L16 AND E386+OLD,NT,PFT,RT
L30          1688 S L16 AND CHROMATOG?
L31          1864 S L17-L30
L32           324 S L31 AND PH
                E PH/CT
L33            33 S L31 AND E3+OLD,NT,PFT,RT
                E E3+ALL
L34            25 S L31 AND E8,E9,E7+NT
L35           330 S L32-L34

     FILE 'REGISTRY' ENTERED AT 11:20:08 ON 04 AUG 2004
L36             1 S 7439-95-4
                E MG/MF
L37            82 S E3
L38             1 S 7440-70-2
                E CA/MF
L39           113 S E3
L40             1 S 9002-98-6
L41             1 S 26913-06-4
L42             1 S 151-56-4
L43          1543 S 151-56-4/CRN
L44          1542 S L43 NOT L40

     FILE 'HCAPLUS' ENTERED AT 11:23:33 ON 04 AUG 2004
L45             8 S L36,L37 AND L35
L46             6 S L38,L39 AND L35
L47             4 S L40-L42 AND L35
L48             0 S L44 AND L35
L49            16 S L45-L47
                SEL DN AN 1
L50             1 S L49 AND E1-E3
L51           167 S L35 AND PH(S)(4 OR 5)
L52           157 S L51 NOT L49
L53            23 S L52 AND (BIOCHEM?(L)METHOD?)/SC,SX
L54            60 S L52 AND (MG OR CA OR ?MAGENSIUM? OR ?CALCIUM? OR PEI OR ?ETHY
L55             0 S L53 AND L45
L56           155 S L52 AND (PD<=20020906 OR PRD<=20020906 OR AD<=20020906)
L57            23 S L53 AND L56
                SEL DN AN L57 3 15 16 18 20 22
L58             6 S L57 AND E4-E21
L59            98 S L56 AND PH(3W)(4 OR 5) NOT L57
L60            41 S L56 AND PH()(4 OR 5) NOT L57
L61            23 S L60 AND ACID?
                SEL DN AN 5
L62             1 S E22-E24
L63             8 S L1,L5,L58,L62
L64             8 S L63 AND L1-L35,L45-L63
L65            17 S L53 NOT L64

     FILE 'HCAPLUS' ENTERED AT 11:46:40 ON 04 AUG 2004

=> d l64 all tot

L64  ANSWER 1 OF 8  HCAPLUS  COPYRIGHT 2004 ACS on STN
AN   2004:203578  HCAPLUS
DN   140:213556
ED   Entered STN:  14 Mar 2004
TI   Process for protein extraction
IN   Gehant, Richard L.
```

PA Genentech, Inc., USA
SO U.S. Pat. Appl. Publ., 17 pp.
CODEN: USXXCO
DT Patent
LA English
IC ICM C07K014-245
NCL 530350000; 530417000
CC **9-9 (Biochemical Methods)**
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	US 2004049012	A1	20040311	US 2003-655874	20030905 <--
	WO 2004022581	A1	20040318	WO 2003-US28007	20030905 <--

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRAI US 2002-408653P P 20020906 <--
CLASS

PATENT NO.	CLASS	PATENT FAMILY CLASSIFICATION CODES
US 2004049012	ICM	C07K014-245
	NCL	530350000; 530417000

AB The invention includes a process for **extracting** a target **protein** from **E. coli** cells that includes lowering the **pH** of a whole **E. coli** cell solution to form an acidic solution, disrupting the cells to release the **protein** into the acidic solution, and separating the cellular debris from the released **protein** to obtain a **protein** product enriched in the heterologous target **protein.** The invention also includes addition of a solubility enhancer.

ST process **protein extn**
IT **Escherichia coli**
Extraction
Liquid chromatography
pH
(process for **protein extraction**)
IT **Proteins**
RL: PEP (Physical, engineering or chemical process); PYP (Physical process); PROC (Process)
(process for **protein extraction**)
IT **7439-95-4**, Magnesium, analysis **7440-70-2**, Calcium, analysis **9002-98-6**
RL: ARU (Analytical role, unclassified); ANST (Analytical study)
(solubility enhancers; process for **protein extraction**)

L64 ANSWER 2 OF 8 HCAPLUS COPYRIGHT 2004 ACS on STN
AN **2000:860458** HCAPLUS
DN **134:144077**
ED Entered STN: 08 Dec 2000
TI A turning point in proteome analysis: sample prefractionation via multicompartment electrolyzers with isoelectric membranes
AU Herbert, Ben; Righetti, Pier Giorgio
CS Proteome Systems, Sydney, Australia
SO Electrophoresis (**2000**), 21(17), 3639-3648
CODEN: ELCTDN; ISSN: 0173-0835

PB Wiley-VCH Verlag GmbH
DT Journal
LA English
CC **9-7** (**Biochemical Methods**)
Section cross-reference(s): 6, 10, 13
AB Sample prefractionation, as obtained via multicompartment electrolyzers with isoelec. membranes, greatly enhanced the load ability, resolution and detection sensitivity of two-dimensional (2-D) maps in proteome anal. This was demonstrated with different samples. In an **Escherichia coli** total cell **extract**, anal. by a 2-D map run in a **pH 4-5** gradient showed many more spots when prefractionated, as compared with standard maps available in databases such as SWISS-2DPAGE. Anal. of human plasma in the **pH** 3-6 range showed an increase in the number of highly acidic **proteins** in the fractionated sample compared to whole plasma. With both samples no **protein** precipitation or smears occurred and much larger sample amts. could be loaded upon prefractionation, so that a large number of spots could be visualized by Coomassie staining, which is fully compatible with subsequent matrix assisted laser desorption/ionization-time of flight (MALDI-TOF) anal.
ST **protein** proteome sample prepn electrolyzer isoelec membrane 2D electrophoresis
IT **Proteins**, general, analysis
RL: ANT (Analyte); BSU (Biological study, unclassified); PUR (Purification or recovery); ANST (Analytical study); BIOL (Biological study); PREP (Preparation)
(blood; **protein** sample prefractionation via multicompartment electrolyzers with isoelec. membranes followed by 2D electrophoresis)
IT Membranes, nonbiological
(isoelec.; **protein** sample prefractionation via multicompartment electrolyzers with isoelec. membranes followed by 2D electrophoresis)
IT Blood plasma
Electrolytic cells
Escherichia coli
Sample preparation
(**protein** sample prefractionation via multicompartment electrolyzers with isoelec. membranes followed by 2D electrophoresis)
IT **Proteins**, general, analysis
Proteome
RL: ANT (Analyte); BSU (Biological study, unclassified); PUR (Purification or recovery); ANST (Analytical study); BIOL (Biological study); PREP (Preparation)
(**protein** sample prefractionation via multicompartment electrolyzers with isoelec. membranes followed by 2D electrophoresis)
IT **Gel electrophoresis**
(two-dimensional; **protein** sample prefractionation via multicompartment electrolyzers with isoelec. membranes followed by 2D electrophoresis)
RE.CNT 34 THERE ARE 34 CITED REFERENCES AVAILABLE FOR THIS RECORD
RE
(1) Anon; Electrophoresis 1988, V9, P679
(2) Anon; Electrophoresis 1989, V10, P71
(3) Anon; Electrophoresis 1990, V11, P189
(4) Anon; Electrophoresis 1990, V11, P987
(5) Anon; Electrophoresis 1991, V12, P459
(6) Anon; Electrophoresis 1991, V12, P763
(7) Anon; Electrophoresis 1992, V13, P891
(8) Anon; Electrophoresis 1994, V15, P1347
(9) Anon; Electrophoresis 1994, V15, P307
(10) Anon; Electrophoresis 1995, V16, P1077
(11) Anon; Electrophoresis 1995, V16, P2175
(12) Anon; Electrophoresis 1996, V17, P1653

(13) Anon; Electrophoresis 1996, V17, P811
(14) Anon; Electrophoresis 1997, V18, P1207
(15) Anon; Electrophoresis 1997, V18, P2703
(16) Anon; Electrophoresis 1997, V18, P305
(17) Anon; Electrophoresis 1999, V20, P2147
(18) Anon; Electrophoresis 1999, V20, P223
(19) Anon; Electrophoresis 1999, V20, P3481
(20) Anon; Electrophoresis 1999, V20, P643
(21) Anon; Electrophoresis 2000, V21, P1037
(22) Anon; Proteome Research: New Frontiers in Functional Genomics 1997, P1
(23) Anon; http://ionsource.com/
(24) Chevallet, M; Electrophoresis 1998, V19, P1901 HCAPLUS
(25) Herbert, B; Electrophoresis 1998, V19, P845 HCAPLUS
(26) Molloy, M; Eur J Biochem 2000, V267, P2871 HCAPLUS
(27) Rabilloud, T; Anal Chem 2000, V72, P48A HCAPLUS
(28) Righetti, P; Immobilized pH Gradients: Theory and Methodology 1990, P1 HCAPLUS
(29) Righetti, P; J Chromatogr 1989, V475, P293 HCAPLUS
(30) Righetti, P; J Chromatogr 1989, V470, P337 HCAPLUS
(31) Righetti, P; J Chromatogr 1990, V500, P681 HCAPLUS
(32) Sarioglu, H; Electrophoresis 2000, V21, P2209 HCAPLUS
(33) Wilkins, M; J Mol Biol 1999, V289, P645 HCAPLUS
(34) Williams, K; Electrophoresis 1998, V19, P1853

L64 ANSWER 3 OF 8 HCAPLUS COPYRIGHT 2004 ACS on STN
AN **1994:624994** HCAPLUS
DN **121:224994**
ED Entered STN: 12 Nov 1994
TI A novel pullulanase that is thermostable under **acid** conditions and cloning and expression of the gene encoding it
IN DeWeer, Philippe; Amory, Antoine
PA Solvay et Cie., Belg.
SO Eur. Pat. Appl., 61 pp.
CODEN: EPXXDW
DT Patent
LA French
IC ICM C12N015-56
ICS C12N015-75; C12N009-44; C12P019-16; C12N001-21; C12N001-20
ICI C12N001-21, C12R001-10; C12N001-20, C12R001-07
CC 7-2 (Enzymes)
Section cross-reference(s): 10, 17
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE	
PI	EP 605040	A1	19940706	EP 1993-203593	19931220	<--
	EP 605040	B1	19990811			
	R: AT, BE, CH, DE, DK, ES, FR, GB, IT, LI, NL, PT					
	BE 1006483	A3	19940913	BE 1992-1156	19921228	<--
	BE 1007313	A3	19950516	BE 1993-744	19930715	<--
	BE 1007723	A6	19951010	BE 1993-1278	19931119	<--
	AT 183236	E	19990815	AT 1993-203593	19931220	<--
	ES 2137222	T3	19991216	ES 1993-203593	19931220	<--
	FI 9305900	A	19940629	FI 1993-5900	19931228	<--
	CN 1090325	A	19940803	CN 1993-121736	19931228	<--
	CN 1061089	B	20010124			
	JP 06217770	A2	19940809	JP 1993-337202	19931228	<--
	CA 2112028	AA	19940629	CA 1993-2112028	19931229	<--
	CA 2112028	C	20030311			
	AU 9352759	A1	19940707	AU 1993-52759	19931230	<--
	AU 686574	B2	19980212			
	US 5721127	A	19980224	US 1995-474140	19950607	<--
	US 5721128	A	19980224	US 1995-477630	19950607	<--
	US 5731174	A	19980324	US 1995-472293	19950607	<--

	US 5736375	A	19980407	US 1995-474545	19950607 <--
	US 6074854	A	20000613	US 1997-996733	19971223 <--
	AU 9864831	A1	19980730	AU 1998-64831	19980511 <--
PRAI	BE 1992-1156	A	19921228 <--		
	BE 1993-744	A	19930715 <--		
	BE 1993-1278	A	19931119 <--		
	US 1993-174893	B1	19931228 <--		
	US 1995-472293	A1	19950607 <--		

CLASS

PATENT NO.	CLASS	PATENT FAMILY CLASSIFICATION CODES
EP 605040	ICM	C12N015-56
	ICS	C12N015-75; C12N009-44; C12P019-16; C12N001-21; C12N001-20
	ICI	C12N001-21, C12R001-10; C12N001-20, C12R001-07

AB A novel pullulanase that is heat-stable at **acid pHs** is obtained from Bacillus and the gene encoding it is cloned and expressed for manufacture of the enzyme for processing polysaccharides. The enzyme has a temperature optimum of 55-65° at **pH 4.**3 and retains >80% of its activity in the **pH** range 3.8-**4.**9. An isolate of Bacillus deramificans capable of hydrolyzing a pullulan derivative at 37°; the strain (B. deramificans T89.117D) was not itself heat-tolerant. The enzyme accumulated in the medium and was purified 10-fold (32% yield) from cultures grown on yeast **extract**/potato starch medium by centrifugation, heat treatment, acetone precipitation, and ion-exchange **chromatog.** The gene was cloned by expression from a Sau3A partial bank in pBR322. The cloned gene was expressed in a Bacillus licheniformis host from which the alkaline **proteinase** gene had been deleted using either an autonomously replicating or integrating plasmid.

ST pullulanase **acid** thermostable Bacillus gene

IT **pH**
(**acid**, pullulanase of Bacillus resistant to high temps. in, purification of and cloning and expression of gene for)

IT Bacillus
Bacillus licheniformis
(alkaline protease-deficient, expression in, of gene for thermostable pullulanase of Bacillus deramificans)

IT Deoxyribonucleic **acid** sequences
(for pullulanase of Bacillus deramificans)

IT Gene, microbial
RL: BIOL (Biological study)
(for thermostable pullulanase of Bacillus deramificans, cloning and expression of)

IT **Protein** sequences
(of pullulanase of Bacillus deramificans)

IT Plasmid and Episome
(pUBDEBRA1, pUBDEBRA11DNSI, gene for thermostable pullulanase of Bacillus deramificans on, cloning and expression in **Escherichia coli** and Bacillus licheniformis of)

IT Bacillus deramificans
(pullulanase of, thermostability at **acid pH** of, purification of, cloning and expression of cDNA for)

IT Saccharification
(enzymic, of starch, **acid** thermostable pullulanase of Bacillus deramificans for, cloning and expression of gene in relation to)

IT Temperature effects, biological
(heat, pullulanase of Bacillus resistant to, at **acid pH**, purification of and cloning and expression of gene for)

IT 9001-92-7, **Proteinase**
RL: BIOL (Biological study)
(alkaline, Bacillus deficient in, expression in, of gene for thermostable

pullulanase of Bacillus deramificans)
IT 157260-41-8, [1-20] Pullulanase (Bacillus deramificans clone pUBDEBRA1)
RL: PRP (Properties); BIOL (Biological study)
(amino **acid** sequence of)
IT 158131-04-5 158131-05-6
RL: BIOL (Biological study)
(amino **acid** sequence of and cloning and expression of gene for, thermostability at **acid pH** of)
IT 158131-06-7 158131-07-8
RL: BIOL (Biological study)
(nucleotide sequence and cloning and expression in Bacillus and **Escherichia coli** of)
IT 9075-68-7P, Pullulanase
RL: PREP (Preparation)
(of Bacillus deramificans, thermostability at **acid pH** of, purification of, cloning and expression of gene for)
IT 9005-25-8, Starch, reactions
RL: RCT (Reactant); RACT (Reactant or reagent)
(saccharification of, thermostable pullulanase of Bacillus deramificans for, cloning and expression of gene in relation to)

L64 ANSWER 4 OF 8 HCAPLUS COPYRIGHT 2004 ACS on STN
AN **1990:568312** HCAPLUS
DN **113:168312**
ED Entered STN: 09 Nov 1990
TI Interaction between heat shock **protein** DnaK and recombinant staphylococcal **protein** A
AU Hellebust, Halldis; Uhlen, Mathias; Enfors, Sven Olof
CS Dep. Biochem. Biotechnol., R. Inst. Technol., Stockholm, S-10044, Swed.
SO Journal of Bacteriology (**1990**), 172(9), 5030-4
CODEN: JOBAAY; ISSN: 0021-9193
DT Journal
LA English
CC **9-3** (**Biochemical Methods**)
Section cross-reference(s): 6
AB When a **protein** derived from the IgG-binding domains of staphylococcal **protein** A was expressed in **Escherichia coli** and recovered from cell **extract** by IgG affinity **chromatog.**, the 69-kilodalton heat shock **protein** DnaK was copurified. DnaK could be selectively eluted from the IgG column by ATP or by lowering the **pH** to **4.7**. **Protein** A could subsequently be eluted by lowering the **pH** to 3.2. Thus, this procedure allows a one-step purification of both DnaK and **protein** A from cell **extract** In vitro expts. with pure DnaK and **protein** A revealed that DnaK did not interfere with the IgG-binding properties of **protein** A but associated with its unfolded C-terminal in a salt-resistant manner. In addition, a specific interaction between DnaK and denaturated casein was found.
ST affinity **chromatog protein** DnaK A; heat shock **protein** DnaK **protein** A
IT Caseins, biological studies
RL: PRP (Properties)
(interaction of, with DnaK)
IT **Proteins**, specific or class
RL: ANST (Analytical study)
(A, purification of DnaK and, by affinity **chromatog.**)
IT Immunoglobulins
RL: ANST (Analytical study)
(G, immobilized, in DnaK and **protein** A purification)
IT Deoxyribonucleic acid formation factors
RL: ANST (Analytical study)
(gene dnaK, purification of **protein** A in, by affinity **chromatog.**)

IT 56-65-5, ATP, biological studies
RL: BIOL (Biological study)
(heat shock **protein** emission from IgG column by)

L64 ANSWER 5 OF 8 HCAPLUS COPYRIGHT 2004 ACS on STN
AN **1989:191250** HCAPLUS
DN **110:191250**
ED Entered STN: 26 May 1989
TI Interleukin-1 and its purification from recombinant **Escherichia coli**
IN Kronheim, Shirley R.
PA Immunex Corp., USA
SO PCT Int. Appl., 24 pp.
CODEN: PIXXD2
DT Patent
LA English
IC ICM C07K015-00
ICS C07K003-20; C07K003-28; C12P021-00
CC 16-2 (Fermentation and Bioindustrial Chemistry)
Section cross-reference(s): 1, **9**

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 8801624	A1	19880310	WO 1987-US2140	19870827 <--
	W: AU, DK, JP, KR				
	RW: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE				
	US 4801686	A	19890131	US 1986-908426	19860904 <--
	AU 8780228	A1	19880324	AU 1987-80228	19870827 <--
	AU 598861	B2	19900705		
	EP 315650	A1	19890517	EP 1987-906498	19870827 <--
	EP 315650	B1	19930310		
	R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE				
	JP 02500562	T2	19900301	JP 1987-505881	19870827 <--
	JP 2525023	B2	19960814		
	AT 86635	E	19930315	AT 1987-906498	19870827 <--
PRAI	US 1986-908426		19860904 <--		
	EP 1987-906498		19870827 <--		
	WO 1987-US2140		19870827 <--		

CLASS

PATENT NO.	CLASS	PATENT FAMILY CLASSIFICATION CODES
WO 8801624	ICM	C07K015-00
	ICS	C07K003-20; C07K003-28; C12P021-00

AB A method of purifying recombinant interleukin-1 (rIL-1) from transformed **E. coli** by comprises (1) suspending the cells in a buffered medium of **pH** 1-5; (2) disrupting the cells; and (3) **extracting** the **proteins.** Transformed **E. coli** cells were shake-cultured in a super induction medium (Mott et al., 1985) plus antibiotics at 30°, derepressed at a cell d. corresponding to A600 = 0.05 by elevating the temperature to 42° and harvested 16 h after the temperature shift. After centrifugation, the cell pellets (from 2.5-L culture) were suspended in Tris-HCl buffer (**pH** 8.0) containing 5 mM EDTA and 1 mM phenylmethylsulfonyl fluoride, frozen, thawed incubated in sodium citrate buffer containing EDTA and lysozyme for 60 min at 37°, again frozen, thawed, and centrifuged to get supernatant. The supernatant was suspended at **pH** 2.8, subjected to **chromatog.** on SPS C-25, DEAE-Sephacel, **Ph** Sepharose CL-4B, concentrated, and rechromatographed on SPS C-25 to obtain 16.8 **mg** rIL-1α with a sp. activity of 6.5 + 108 units rIL-1α/ **mg** and a endotoxin content of 60 pg endotoxin/μg IL-1α. Sep., **E** . **coli** was suspended in **pH** 3.9 buffer, and processed to obtain 85.2 **mg** rIL-1β/mL culture with a sp. activity of

1.95 + 108 units/**mg** and 36 pg endotoxin/μg rIL-1β
by subjecting the rIL-1β containing **exts.** to **chromatog**
. on SPS C-25, DEAE-Sephacel, and Procion Red agarose.
ST recombinant interleukin 1 purifn Escherichia; acidic **extn**
recombinant interleukin 1 purifn
IT Lymphokines and Cytokines
RL: BIOL (Biological study)
(interleukin 1α, purification of recombinant, from **Escherichia coli**)
IT Lymphokines and Cytokines
RL: BIOL (Biological study)
(interleukin 1β, purification of recombinant, from **Escherichia coli**)
IT 9012-36-6D, Agarose, reaction product with Procion Red 9013-34-7,
DEAE-Sephacel 61840-62-8 64940-65-4D, Procion Red, reaction product
with agarose 69106-59-8, Phenyl Sepharose CL-4B
RL: BIOL (Biological study)
(recombinant interleukin-1 purification on)

L64 ANSWER 6 OF 8 HCAPLUS COPYRIGHT 2004 ACS on STN
AN **1986:65233** HCAPLUS
DN **104:65233**
ED Entered STN: 08 Mar 1986
TI Two-dimensional polyacrylamide gel electrophoresis of **Escherichia coli proteins** with Pharmalytes
AU Johansson, Karl Erik
CS Mycoplasmalab., Natl. Vet. Inst., Uppsala, S-750 07, Swed.
SO Protides of the Biological Fluids (**1985**), 33, 475-8
CODEN: PBFPA6; ISSN: 0079-7065
DT Journal
LA English
CC **9-7** (**Biochemical Methods**)
AB Cell **exts.** of **E. coli** were analyzed by
2-dimensional polyacrylamide gel electrophoresis (0'Farrell technique)
with isoelec. focusing in the first and SDS polyacrylamide gel
electrophoresis in the second dimension. Different mixts. of Pharmalytes
were used to study the influence of the carrier ampholyte composition on the
shape of the **pH** gradient and the resolution in the final
2-dimensional **protein** pattern, which was developed by a Ag
staining procedure. Optimal resolution was obtained with a mixture of the
pH ranges (2.5-5), (5-8) and (3-10)
in the proportions (2:1:1). This cocktail gave a shallow **pH**
gradient in the acid region with the **protein** spots evenly
distributed over the whole gel plate.
ST Escherichia **protein** Pharmalyte gel electrophoresis; two
dimensional polyacrylamide electrophoresis **protein**
IT **Proteins**
RL: PROC (Process)
(electrophoresis of, 2-dimensional polyacrylamide gel, of **Escherichia coli**)
IT **Escherichia coli**
(**proteins** of, 2-dimensional polyacrylamide gel
electrophoresis of)
IT **Electrophoresis and Ionophoresis**
(**gel, two-dimensional**, of
proteins, of **Escherichia coli** on
polyacrylamide)
IT 70852-56-1
RL: ANST (Analytical study)
(in two-dimensional polyacrylamide gel electrophoresis of **Escherichia coli proteins**)

L64 ANSWER 7 OF 8 HCAPLUS COPYRIGHT 2004 ACS on STN

AN **1979:199738** HCAPLUS
DN **90:199738**
ED Entered STN: 12 May 1984
TI **Protein** S1 from **Escherichia coli** ribosomes: an improved isolation procedure and shape determination by small-angle x-ray scattering
AU Labischinski, Harald; Subramanian, Alap Raman
CS Inst. Kristallogr., Freie Univ. Berlin-Dahlem, Berlin, Fed. Rep. Ger.
SO European Journal of Biochemistry (**1979**), 95(2), 359-66
CODEN: EJBCAI; ISSN: 0014-2956
DT Journal
LA English
CC **9-2** (**Biochemical Methods**)
Section cross-reference(s): 6
AB A 3-step procedure was developed for the large-scale purification of ribosomal **protein** S1 (and **protein** A) of **E. coli**, starting with **proteins extracted** by 2M LiCl/4M urea. The method involves: (a) **chromatog.** on DEAE-cellulose at **pH 5.5** to isolate very acidic **proteins**; (b) gel filtration of the eluted **proteins** on Sephadex G 25; and (c) gradient elution on a 2nd DEAE-cellulose column of the high-mol.-weight fraction from Sephadex. **Protein** S1 then was studied in solution by small-angle x-ray scattering, and the following parameters were obtained. The radius of gyration R = 8.0 nm, largest diameter D = 28 nm, mol. weight = (8-9) + 104. The data also yeilded (with the assumption of a rigid particle with almost constant electron d.) 2 radii of gyration of cross-section Rq1 = 2.5 nm and Rq2 = 1.05 nm and mol. volume = 140 nm3. The exptl. scattering curve of S1 was compared with the theor. scattering curves for several rigid triaxial homogeneous bodies, and the closest fit was given by that of a flat elliptical cylinder with the dimensions of 4.5 nm and 0.88 nm for the 2 semiaxes and 26.5 nm for height. The results from the present x-ray scattering studies and those from limited proteolytic digestion of **protein** S1 (Suryanarayana, T; Subramanian A. R., 1979) support the notion that the structure of **protein** S1 is organized into 2 distinct subdomains within its elongated overall shape.
ST Escherichia ribosome **protein** S1 A; x ray **protein** S1
IT Chains, chemical
(conformation of, of ribosomal **protein** S1 of **Escherichia coli**)
IT Radius of gyration
Water of hydration
(of ribosomal **protein** S1, of **Escherichia coli**)
IT Ribosome
(**protein** S1 of, of **Escherichia coli**, isolation and shape determination of)
IT **Escherichia coli**
(ribosomal **protein** S1 of, isolation and shape determination of)
IT **Proteins**
RL: PROC (Process)
(A, of ribosome of **Escherichia coli**, isolation of)
IT **Proteins**
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); BIOL (Biological study); OCCU (Occurrence)
(S1, of **Escherichia coli** ribosome, isolation and shape determination of)

L64 ANSWER 8 OF 8 HCAPLUS COPYRIGHT 2004 ACS on STN
AN **1972:11781** HCAPLUS
DN **76:11781**
ED Entered STN: 12 May 1984
TI Ribosomal **proteins.** Isolation of the **proteins** from

30S ribosomal subunits of **Escherichia coli**
AU Hindennach, Ingrid; Stoeffler, Georg; Wittmann, Heinz G.
CS Max-Planck-Inst. Mol. Genet., Berlin-Dahlem, Fed. Rep. Ger.
SO European Journal of Biochemistry (**1971**), 23(1), 7-11
CODEN: EJBCAI; ISSN: 0014-2956
DT Journal
LA English
CC **9** (**Biochemical Methods**)
AB All 21 ribosomal **proteins** from 30S subunits of **E. coli** were isolated in a pure state by the following methods: separation of subunits by zonal centrifugation in B XV rotors, **extraction** of **proteins**, CM-cellulose **chromatog.** with pyridine formate gradients, gel filtration on Sephadex G-100 and, in some cases, preparative polyacrylamide block electrophoresis. The purity of isolated **proteins** was 97-100%, shown by disc electrophoresis at **pH 4.5** in urea, by 2-dimensional polyacrylamide electrophoresis, by dodecyl sulfate gel electrophoresis and by cellulose acetate gel electrophoresis. The yield of the isolated **proteins** was 7-110 **mg** depending on the **protein** and the number of purification steps. Fractionation of total 30S **proteins** with (NH4)2SO4 is recommended for quick isolation of **proteins** S15 and S20 in large quantities.
ST ribosome **protein** Escherichia
IT Ribosome
(30S, **proteins** isolation from, of **Escherichia coli**)
IT **Proteins**
RL: PROC (Process)
(of ribosomes of **Escherichia coli**, isolation of)
IT **Escherichia coli**
(**proteins** of ribosomes of, isolation of)

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http://www.stn-international.de/archive/stnews/news0104.pdf <<<

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L84 ANSWER 1 OF 11 WPIX COPYRIGHT 2004 THOMSON DERWENT on STN
AN **2004-419682** [39] WPIX
DNN **N2004-333124** DNC **C2004-157576**
TI Enhancing the solubilization of a **proteinaceous** macromolecule for obtaining medically, significant **proteins** from microorganisms, comprises incubating biological sample in a solubilization reagent at acidic **pH.**
DC B04 D16 S03
IN HERBERT, B R
PA (PROT-N) PROTEOME SYSTEMS INTELLECTUAL PROPERTY P
CYC 107
PI WO 2004041848 A1 20040521 (200439)* EN 34 C07K001-02 <--
RW: AT BE BG BW CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE LS LU MC MW MZ NL OA PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NI NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW
ADT WO 2004041848 A1 WO 2003-AU1448 20031103
PRAI AU 2002-952533 20021104
IC ICM **C07K001-02**
ICS **C07K001-14; C07K001-24; C07K001-26; C07K001-28; C07K001-30; C07K001-36**
AB WO2004041848 A UPAB: 20040621
NOVELTY - Enhancing (M1) the solubilization of a **proteinaceous** macromolecule in a biological sample without inducing substantial acid hydrolysis of the **proteinaceous** macromolecule, comprises incubating the biological sample in a solubilization reagent at a **pH** between 1-6.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following:

(1) a kit for enhancing solubilization of a **proteinaceous** macromolecule in a biological sample without inducing substantial acid hydrolysis of the **proteinaceous** macromolecule, comprising a solubilization reagent to solubilize at least one macromolecule in a biological sample, where the solubilization reagent has a **pH** of 1-6 and optionally comprising directions to solubilize and/or recover a macromolecule in a biological sample, and/or directions to resolve a macromolecule in a biological sample;

(2) a **proteinaceous** macromolecule solubilized by (M1);

(3) use of an acidic reagent having a **pH** of 1-6 in the preparation of an solubilization reagent solution for use in solubilizing a **proteinaceous** macromolecule from a biological sample, without inducing substantial acid hydrolysis of the **proteinaceous** macromolecule.

USE - (M1) is useful for enhancing the solubilization of at least one **proteinaceous** macromolecule in a biological sample without inducing substantial acid hydrolysis of the **proteinaceous** macromolecule (claimed). (M1) is useful for **extraction** of **proteinaceous** macromolecule from a biological sample such as microorganism sample (bacterial, yeast, or fungal), an insect cell sample (exoskeleton sample), plant cell sample or seed, an animal cell sample or tissue sample (skin and hair sample), or their fractions. (M1) is useful for obtaining medically, agriculturally and environmentally significant **proteins** from microorganisms.

ADVANTAGE - (M1) enhances solubilization of **proteinaceous** macromolecule in a biological sample without inducing substantial acid hydrolysis of the macromolecule (claimed). (M1) minimizes removal of a sugar or sugars from the backbone of the **protein** during the solubilization. Cell debris are more easily removed during the **purification** process, as the acidic conditions preferably prevent solubilization of cell membrane or cell wall. (M1) does not require to precipitate the **protein** prior to solubilization. (M1) preferably enhances solubilization of alkaline **proteins.**

Dwg.0/8

FS CPI EPI

FA AB; DCN

MC CPI: B03-F; B04-L05C; B04-N01; B04-N02; B04-N03; B04-N04; B05-B02A3; B10-C02; B10-C04C; B10-C04E; **B11-B**; B11-C08D; B11-C08E; B12-K04; D05-H09; D05-H13

EPI: S03-E14H5

TECH UPTX: 20040621

TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Method: In (M1), the solubilization reagent has a **pH** of 1-6. The biological sample is incubated in a solubilization reagent at a **pH** of 2-**5**, 3-4, or 2-3. The **proteinaceous** macromolecule is solubilized in the presence of an aqueous acidic reagent chosen from an organic acid solution, inorganic acid solution, acidic buffer, amino acid solution or their mixtures. The organic acid is chosen from ascorbic acid, carboxylic acid and polycarboxylic acid, or their derivative or mixtures. The carboxylic acid is chosen from formic acid, acetic acid, propionic acid, butyric acid, valeric acid, and benzoic acid, or their derivative mixtures. The polycarboxylic acid is chosen from oxalic acid and citric acid or its derivative or mixture. The inorganic acid is chosen from phosphoric acid, and orthophosphoric acid or their derivative or mixture. The acidic buffer comprises a citrophospho buffer. The solubilization reagent comprises a chaotropic agent. The solubilization reagent comprises a detergent. The biological sample is subjected to physical or chemical mode to disrupt the biological sample. (M1) further involves recovering the at least one solubilized **proteinaceous** macromolecule. The solubilized **proteinaceous** macromolecule is recovered by performing a process involving precipitating the at least one solubilized macromolecule. The solubilized **proteinaceous** macromolecule is recovered by performing a process involving precipitating and resuspending the **protein** precipitate. (M1) further involves reducing and alkylating the resuspended **protein** precipitate. (M1) involves (i) subjecting the biological sample to a physical or chemical mode to disrupt the biological sample and incubating the biological sample in the presence of a reagent at a **pH** between 1-6 to thus solubilize at least one **proteinaceous** macromolecule in the biological sample, and (ii) performing one or more processes chosen from (a) recovering the solubilized **proteinaceous** macromolecule by performing a process involving precipitating one or more **proteins** in the **extract** to thus precipitate at least the solubilized **proteinaceous** macromolecule and resuspending the precipitated **proteinaceous** macromolecule, (b) reducing and alkylating the solubilized **proteinaceous** macromolecule or the resuspended **proteinaceous** macromolecule, and (c) subjecting the solubilized **proteinaceous** macromolecule or the resuspended **proteinaceous** macromolecule or the reduced and alkylated **proteinaceous** macromolecule to a resolving mode for a time and under conditions sufficient to resolve the **proteinaceous** macromolecule from other macromolecules present in the biological sample and then identifying the resolved **proteinaceous** macromolecule. (M1) optionally involves performing step (i) to produce a **proteinaceous extract** and incubating the **extract** in the same way as in step (i), and performing step (ii) as described above. The resolving mode comprises a proteomic technique chosen from

two-dimensional electrophoresis, one-dimensional electrophoresis, HPLC and liquid **chromatography**-mass spectrometry (LC-MS) or their combination. (M1) further involves digesting the resolved macromolecule, and identifying the digested macromolecule by mass-spectrometry. The macromolecule is digested by at least one proteolytic enzyme.

ABEX UPTX: 20040621

EXAMPLE - Dry or wet **Escherichia coli**, Saccharomyces cerevisiae, Bacillus subtilis or Trichoderma harzianum cells were solubilized by ultrasonication in 7 M urea, 2 M thiourea, 1% C7Bz0 and either 80 mM citric acid (**pH 4**), 80 mM ascorbic acid (**pH 4**), orthophosphoric acid (**pH** 3) or orthophosphoric acid (**pH** 2). In some cases acidic sample preparations were done with 1 mM phenylmethanesulfonylfluoride (PMSF) and 0.1% protease inhibitor cocktail. The sample was maintained below 35degreesC during the sonication. The **extraction** solution was kept on ice and ultrasonicated for 4 bursts of 30 seconds with 1 minutes cooling between bursts. After **centrifugation** at 40000 g for 20 minutes, the supernatant **protein** was precipitated by the addition of 5 volumes of room temperature acetone. The precipitated **proteins** were recovered by **centrifugation** at 5000 g for 10 minutes and resuspended in Sigma ProteoPrep (RTM; Prot-Tot) **extraction** solution No.4, **pH 10.4.** This solution was 7 M urea, 2 M thiourea, 1% C7Bz0 and 40 mM Tris. The now alkaline **protein extract** was reduced with 5 mM tributylphosphine (TBP) and alkylated with 10 mM acrylamide monomer in a single 2 hour step. The excess acrylamide was then quenched with the addition of 10 mM dithiothreitol (DTT), and the sample aliquoted prior to storage at either-20degreesC or -80degreesC. The final **extract** contained between 2-3 mg/ml of **protein.**

L84 ANSWER 2 OF 11 WPIX COPYRIGHT 2004 THOMSON DERWENT on STN
AN **2004-293803** [27] WPIX
DNC **C2004-112409**
TI **Extracting** a **target protein** from **Escherichia coli** cells comprises lysing the cells under acidic conditions, preferably with the addition of a solubility enhancer, and **separating** the **target protein** from cellular debris.
DC A89 B04 D16
IN **GEHANT, R L**
PA (GETH) GENENTECH INC
CYC 104
PI US 2004049012 A1 20040311 (200427)* 17 C07K014-245 <--
WO 2004022581 A1 20040318 (200427) EN C07K001-36 <--
RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE LS LU MC MW MZ NL OA PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PG PH PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG UZ VC VN YU ZA ZM ZW
ADT US 2004049012 A1 **Provisional US 2002-408653P 20020906**, US 2003-655874 20030905; WO 2004022581 A1 **WO 2003-US28007 20030905**
PRAI **US 2002-408653P 20020906**; US 2003-655874 20030905
IC ICM **C07K001-36; C07K014-245**
AB US2004049012 A UPAB: 20040426
NOVELTY - **Extraction** of a **target protein** from **Escherichia coli** cells comprises:
(a) lowering the **pH** of a solution containing whole **E. coli** cells expressing a heterologous **target protein**;
(b) lysing the cells; and

(c) **separating** cellular debris from the released **target protein** to obtain a product enriched in the **target protein.**

DETAILED DESCRIPTION - **Extraction** of a **target protein** from **Escherichia coli** cells comprises:

(a) lowering the **pH** of a solution containing whole **E. coli** cells expressing a heterologous **target protein**;

(b) lysing the cells; and

(c) **separating** cellular debris from the released **target protein** to obtain a product enriched in the **target protein.**

The acidification of the solution decreases biomass-biomass interactions and/or biomass-resin interactions, and increases the moisture content of a flocculent in the released **protein** solution.

An INDEPENDENT CLAIM is also included for a **protein** product produced by the method.

USE - The method is useful for **extracting** a **target protein** from **Escherichia coli** cells (claimed).

ADVANTAGE - The biomass-biomass interaction and/or biomass- resin interaction of the disrupted cell solution is reduced as compared with solution at a non-acidic **pH.** The moisture content of a flocculent is greater as compared with the non-acidic solution. The method serves to increase the solubility and purity of the **target protein** at lower cost.

Dwg.0/8

FS CPI

FA AB; DCN

MC CPI: A12-L04; **B04-C01**; B04-C03; B05-A01B; **B11-B**; D05-H13; D05-H17

TECH UPTX: 20040426

TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Method: The **pH** is lowered to 4 - 5 (preferably 4 - 4.5, especially 4). The **separation** comprises **centrifugation.** The method involves addition of at least one solubility enhancer prior to or contemporaneous with **pH** lowering. The method further involves **purification** of heterologous **target protein** from the **protein** product by column **chromatography** (preferably expanded bed **chromatography**).

TECHNOLOGY FOCUS - INORGANIC CHEMISTRY - Preferred Components: The solubility enhancer is a divalent cation (10 - 150 mM) (preferably magnesium or calcium) and/or **polyethyleneimine** (PEI) (0.2 - 0.3 vol./vol.% of 50 wt/vol.% solution).

ABEX UPTX: 20040426

EXAMPLE - **E. coli** cells containing a transgene expressing anti-vascular endothelial growth factor (VEGF) antibody fragment were pre-conditioned before homogenization by lowering the **pH** of the cell solution to 4 via addition of citric acid (60 nM). The solubility enhancer, MgSO4 (120 mM) was added to the solution. The cells were disrupted by 4 pass homogenization at 8000 psig and 2 - 8 degrees C. After homogenization water (5 volumes) was added and the homogenate was passed through EBC inlet screen, with the flow rate of 25 ml/min. Control cells were similarly disrupted by 4 pass homogenization but with post homogenization conditioning at **pH** 4 and without addition of MgSO4. Acid was added to the homogenized cells to **pH** 4 as well as MgSO4 (120 mM) and water (2 volumes). The resulting material was **centrifuged** to pellet insoluble material. The control supernatant was applied to EBC inlet screen. The results demonstrated that pre-conditioning of **E. coli** at **pH** 4 and in presence of MgSO4 reduced biomass-biomass interaction by reducing flocculation of **protein** solution.

L84 ANSWER 3 OF 11 WPIX COPYRIGHT 2004 THOMSON DERWENT on STN
AN **2003-731381** [69] WPIX
DNC **C2003-201206**
TI New xylose isomerase **polypeptide isolated** from Thermotoga neapolitana and evolved using random mutagenesis and screening, useful in production of fructose syrup especially industrially to produce high fructose corn syrup.
DC B04 D16 D17
IN SRIPRAPUNDH, D; VIEILLE, C; ZEIKUS, J G
PA (UNMS) UNIV MICHIGAN STATE
CYC 102
PI WO 2003062387 A2 20030731 (200369)* EN 70 C12N000-00
RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE LS LU MC MW MZ NL OA PT SD SE SI SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SC SD SE SG SK SL TJ TM TN TR TT TZ UA UG UZ VC VN YU ZA ZM ZW
US 2003166172 A1 20030904 (200369) C12P019-02
AU 2003219671 A1 20030902 (200422) C12N000-00
ADT WO 2003062387 A2 WO 2003-US1681 20030121; US 2003166172 A1 Provisional US 2002-350930P 20020123, Provisional US 2002-428064P 20021121, US 2003-348552 20030121; AU 2003219671 A1 AU 2003-219671 20030121
FDT AU 2003219671 A1 Based on WO 2003062387
PRAI US 2002-428064P 20021121; US 2002-350930P 20020123; US 2003-348552 20030121
IC ICM C12N000-00; C12P019-02
ICS C07H021-04; C12N005-06; C12N009-92; C12P021-02; C12Q001-68
AB WO2003062387 A UPAB: 20031027
NOVELTY - A **polypeptide** having at least 80 % identity to amino acids 2-444 of one of a selection of 444 amino acid sequences (I)-(V), fully defined in the specification, for a Thermotoga neapolitana xylose isomerase **polypeptide**, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for:

(1) **isolated** polynucleotide:

(i) encoding **polypeptide** as above;

(ii) encoding **polypeptide** having at least 80 % identity to amino acids 2-444 of one of sequence (I)-(V) and preferably having sequence with at least 95 % identity to nucleic acids 616-1950 of one of 2522 bp sequences (VI)-(X);

(iii) complementary to polynucleotide as in (ii), especially comprising at least 50 nucleotides; and

(iv) hybridizing to polynucleotide as in (ii), especially comprising 50-200 nucleotides;

(2) fusion **protein** comprising **polypeptide** as above operably linked to a second **polypeptide**, with cleavage site between **polypeptides**;

(3) polynucleotide encoding fusion **protein**;

(4) nucleic acid construct comprising polynucleotide as in (1)(i) operably linked to one or more control sequences directing **polypeptide** production in expression host;

(5) vector comprising polynucleotide as in (1)(i), especially expression vector comprising construct as in (4), promoter and transcription and translation stop signs;

(6) host cell comprising vector as in (4); and

(7) antibody selectively binding **polypeptide.**

USE - The methods are useful for producing fructose syrup, and xylose isomerase. Fructose syrup is produced by contacting a preparation containing glucose with the **polypeptide.** Preferably, preparation comprises 30-55 weight % (or 35-99 weight %) dry glucose, reaction temperature is 50-100 (especially 60-90) deg. C, **pH** is 4.5-8 (especially 5.2-8.2), and reaction is a continuous, fixed-bed reactor process and is

carried out in presence of at least one of Mg2+, Co2+ and Mn2+. Xylose isomerase enzyme may be produced by culturing cells as in (6) and recovering enzyme from culture, or by recovering polynucleotide as in (1)(i); constructing a hybrid plasmid; inserting plasmid into host microorganism that expresses enzyme; and **purifying** enzyme by heating to temperature denaturing most of contaminating **proteins** (all claimed).

The **polypeptide** is useful for producing fructose syrup especially industrially to produce high fructose corn syrup, since it can operate at higher temperatures and lower **pH** (and with greater thermal stability) than existing xylose isomerase enzymes, increasing yield potential and reducing/eliminating non-enzymatic browning. It may also be used in other industrial processes involving action of isomerization enzymes. Polynucleotides may also be used for **polypeptide** detection and to produce antisense sequences useful therapeutically, or probes and primers useful e.g. in detection of identical, similar or complementary sequences (e.g. homologues from other cell types/species).

ADVANTAGE - The **polypeptide** has higher temperature and lower **pH** optima than most xylose isomerase enzymes, and greater thermal stability than an existing xylose isomerase (Gensweet (RTM)) with similar temperature optimum (90 and 85 deg. C respectively), e.g. an order of magnitude at 60 deg. C and **pH** 7 or 5.5.

Dwg.0/20

FS CPI

FA AB; DCN

MC CPI: B04-E02E; B04-E05; B04-E08; B04-F0900E; B04-F10A3E; B04-G01; B04-L0700E; B05-A01B; B05-A03A; B10-A07; **B11-B**; D05-C03F; D05-H11; D05-H12A; D05-H12D1; D05-H12E; D05-H14A1; D05-H14A2; D05-H17A3

TECH UPTX: 20031027

TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preparation (claimed): **Polypeptide** can be produced by culturing host cells of (6) under suitable conditions and recovering **polypeptide** from culture.

Preferred **Polypeptides**: The **polypeptide** preferably catalyzes conversion of glucose to fructose and is preferably 95 % identical to amino acids 2-444 of sequence (I), (II), (III), (IV) or especially (V). It is preferably in immobilized form.

Preferred Vectors: Vector is preferably a plasmid, virus or bacteriophage and preferably contains polynucleotide inserted in proper orientation and correct reading frame so that cell transformed with vector expresses amino acids 2-444 of one of sequence (I)-(V). Polynucleotide is preferably operably linked to a promoter e.g. the tac promoter, cell wall **protein** promoter, phosphoglycerate kinase gene promoter, alcohol dehydrogenase promoter, glycerol kinase PI gene promoter, erythromycin resistance gene EP1 promoter and phage T7 promoter. The vector further comprises transcriptional and translational stop signals.

Preferred Host Cells: Cell is preferably bacterial (especially from genus Bacillus, **Escherichia**, Sacchromyces or Streptomyces) or fungal selected from a yeast cell or a filamentous fungal cell (especially from genus Aspergillus, Fusarium or Trichoderma).

ABEX UPTX: 20031027

SPECIFIC SEQUENCES - Specifically claimed are (I)-(V) comprising defined 444 amino acid sequences encoded by 2522 base pair sequences (VI)-(X) respectively; all sequences are given in the specification.

EXAMPLE - Xylose isomerase gene from Thermotoga neapolitana (TNXI) was cloned, sequenced and overexpressed in **Escherichia coli** and active site engineered by site-directed mutagenesis to increase activity on glucose conventionally. Resulting TNXI Val185Thr (V185T) mutant was more active, more glucose-efficient and as stable as wild-type TNXI and, although highly thermostable and active at 97 degrees C, was poorly active at industrial isomerization temperature (60 degrees C) and

required neutral **pH** for optimal activity. TNXI V185T-encoding gene was therefore used as template for two rounds of random mutagenesis and low temperature/low **pH** activity screening by known methods. Mutants TNXI 3A2 and 1F1 (producing sequences (I) and (V) respectively) were obtained after rounds one and two respectively; 1F1 was the most active at all temperatures and **pH** tested and was also more kinetically stable than TNXI and TNXI V185T.

L84 ANSWER 4 OF 11 WPIX COPYRIGHT 2004 THOMSON DERWENT on STN
AN **2003-505200** [47] WPIX
DNC **C2003-135059**
TI Obtaining a sample of **target** nucleic acid from cells having the **target** nucleic acid and genomic nucleic acid comprises suspending the cells in an aqueous medium to cause the **target** nucleic acid to leak from the cells into the aqueous medium.
DC B04 D16
IN BAKER, M; TAYLOR, M; UPPAL, S
PA (DNAR-N) DNA RES INNOVATIONS LTD
CYC 102
PI WO 2003046177 A1 20030605 (200347)* EN 13 C12N015-10
RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SC SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW
AU 2002343040 A1 20030610 (200419) C12N015-10
ADT WO 2003046177 A1 WO 2002-GB5209 20021120; AU 2002343040 A1 AU 2002-343040 20021120
FDT AU 2002343040 A1 Based on WO 2003046177
PRAI GB 2001-27809 20011120
IC ICM C12N015-10
ICS C12N001-06; C12N001-066
AB WO2003046177 A UPAB: 20030723
NOVELTY - Obtaining a sample of **target** nucleic acid from cells containing the **target** nucleic acid and genomic nucleic acid, comprising suspending the cells in an aqueous medium to cause the **target** nucleic acid to leak from the cells into the aqueous medium.

DETAILED DESCRIPTION - Obtaining a sample of **target** nucleic acid from cells containing the **target** nucleic acid and genomic nucleic acid comprises:

(a) **separating** the cells from culture broth;

(b) suspending the cells in an aqueous medium which causes the **target** nucleic acid to leak from the cells into the aqueous medium; and

(c) obtaining the sample of the nucleic acid from the aqueous medium, where the cells are substantially not lysed during the above steps and substantially retain the genomic nucleic acid within the cells.

USE - The method is useful in **extracting** nucleic acids, particularly in **separating** a non-genomic nucleic acid, such as a cellular vector DNA or RNA, self-replicating satellite nucleic acids or plasmid DNA, from genomic nucleic acids, such as host cell chromosomes and ribosomal RNA.

Dwg.0/5
FS CPI
FA AB; DCN
MC CPI: B04-E01; B05-A01A; B05-A01B; B05-C07; B10-B01B; B10-C04E; **B11-B**; D05-H13
TECH UPTX: 20030723
TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Method: The above method is carried out at a temperature of less than 60degreesC, preferably less than

40degreesC, and in the absence of an electrical field capable of causing cell poration. The cellular **proteins** are substantially retained within the cells. The **target** nucleic acid is non-genomic nucleic acid. The genomic nucleic acid is host cell chromosomal DNA or ribosomal RNA. The **target** nucleic acid sample is a vector, a plasmid, satellite or cosmid DNA or vector RNA. Preferably, the **target** nucleic acid is plasmid DNA. The cells are a gram-negative microorganism, particularly **Escherichia coli.** The aqueous medium is water, a low salt buffer, a salt solution, a divalent or trivalent metal salt solution or a sugar solution. It has a **pH** of about 6-9. The low salt buffer comprises Tris HCl. The Tris HCl buffer has a concentration of about 5-50 mM, has a **pH** of about 8.5, and further comprises EDTA. Alternatively, the low salt buffer comprises potassium acetate/KCl which has a concentration of about 10-30 mM and a **pH** of 4. The salt solution is a sodium chloride solution that has a concentration of about 50-250 mM and/or a **pH** of about 7. The divalent metal ion solution is a CaCl2 solution that has a concentration of about 0.05-1.0 M. The aqueous medium further comprises **Proteinase** K and a non-ionic detergent. In addition, it comprises an RNA nuclease and/or a DNA nuclease and/or a protease. The above method further comprises **purifying** the **target** nucleic acid present in the sample of **target** nucleic acid, and **isolating**, analyzing, amplifying and sequencing the sample of the **target** nucleic acid. The **isolation** of the **target** nucleic acid is by ion exchange, electrophoresis, silica solid phase **extraction**, precipitation, flocculation, filtration, gel filtration, **centrifugation**, alcohol precipitation and/or the use of a charge switch material.

L84 ANSWER 5 OF 11 WPIX COPYRIGHT 2004 THOMSON DERWENT on STN
AN **2003-441202** [41] WPIX
DNC **C2003-116708**
TI Non-specific enrichment of bacteria, useful e.g. for plasmid or nucleic acid recovery, by precipitation with cationic polymer and magnetic carrier without **centrifugation.**
DC A89 B04 D16
IN GRASSL, R; MILLER, S; ROBL, I; SCHUETZ, M; ZANDER, T; DILLER, S
PA (PROF-N) PROFOS AG
CYC 102
PI WO 2003033698 A1 20030424 (200341)* GE 30 C12N015-10
RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW
DE 10149803 A1 20030424 (200341) C12N001-20
DE 10230147 A1 20040115 (200413) C12N013-00
EP 1434864 A1 20040707 (200444) GE C12N015-10
R: AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI SK TR
ADT WO 2003033698 A1 WO 2002-DE3790 20021008; DE 10149803 A1 DE 2001-10149803 20011009; DE 10230147 A1 DE 2002-10230147 20020704; EP 1434864 A1 EP 2002-779144 20021008, WO 2002-DE3790 20021008
FDT DE 10149803 A1 Add in DE 10230147; DE 10230147 A1 Add to DE 10149803; EP 1434864 A1 Based on WO 2003033698
PRAI DE 2002-10230147 20020704; DE 2001-10149803 20011009
IC ICM C12N001-20; C12N013-00; C12N015-10
ICS C12N001-02; C12Q001-24
AB WO2003033698 A UPAB: 20030630
NOVELTY - Method for non-specific enrichment of bacteria (A) comprises:
(a) treating (A)-containing sample with cationic polymer (I) at

acidic **pH**;
(b) addition of magnetic carrier; and
(c) **separation** of the complex formed between (A), (I) and carrier from the sample.
USE - The method is used to **separate** a wide range of bacteria from cultures, e.g. for recovery of plasmids, **proteins**, RNA or genomic DNA, also of live bacteria.
ADVANTAGE - The method provides simple, efficient, rapid (5-10 minutes) and inexpensive (only small amounts of cationic polymer and magnetic beads required) concentration of a wide variety of bacteria. No **centrifugations** are needed, making the method suitable for automation, and many different carriers can be used.
Dwg.0/10
FS CPI
FA AB; DCN
MC CPI: A12-L04; A12-W11L; **B04-C01**; B04-C02C; B04-C02E3; B04-C03; B04-E01; B04-E08; B04-F10; B04-N04; B05-A03A; **B11-B**; D05-C07; D05-C12; D05-H01; D05-H04; D05-H12; D05-H12E
TECH UPTX: 20030630
TECHNOLOGY FOCUS - BIOLOGY - Preferred Materials: (I) is chitosan or polylysine and the carrier is (i) a magnetic pigment or (ii) a magnetic polystyrene bead, preferably with amino, carboxy or hydroxy groups on the surface, particularly of particle size 1-5 microns, especially 1 micron. Preferred Process; Step (a) and/or (b) is an incubation, preferably for 3-10 (especially 5) minutes at room temperature to 40 degrees C and preferably at room temperature. The concentration of polystyrene beads is 1%, used at 1/10-1/70 of the volume of culture, depending on sample size, with a lower concentration for larger samples. Pigment particles are supplied as a 5% suspension at 1/10 of the sample volume. The concentration of (I) is 0.05-100 (most preferably 2.5-50) microg/ml for chitosan or 12.5-150 (most preferably 50) mg/ml for polylysine, with **pH** 3 or lower adjusted with hydrochloric and/or acetic acid or **pH 4** or lower with formic acid. Especially, hydrochloric acid/acetic acid of **pH** 2-3 are used for plasmid recovery and formic acid of **pH 4** for **protein** recovery.
Bacteria are released from the complex formed by treatment with 0.2 M **pH** 8-9.5 Tris buffer and the carrier may then be recycled.
Preferred Bacteria: Bacteria are selected from **Escherichia coli** or species of the genera Bacillus, Proteus, Micrococcus, Staphylococcus or Citrobacter.

TECHNOLOGY FOCUS - POLYMERS - Preferred Polymers: Specified cationic polymers are chitosan and polylysine and carriers are e.g. of polystyrene.
ABEX UPTX: 20030630
WIDER DISCLOSURE - Disclosed is a similar method using an anionic polymer (e.g. dextran sulfate or polyglutamate) at basic **pH.**
EXAMPLE - **Escherichia coli** HB101 was grown overnight in 96-well polypropylene microtiter plates (0.2 ml per well), then mixed with (i) 20 microl of a 125 microg/ml solution of chitosan in 0.5 N formic acid and (ii) 20 microl of a 5% suspension of the magnetic pigment Bayoxide E8706 in phosphate-buffered saline. After 5 minutes incubation at room temperature, a complex of bacteria, polymer and magnetic particles had formed.

L84 ANSWER 6 OF 11 WPIX COPYRIGHT 2004 THOMSON DERWENT on STN
AN **2001-476183** [51] WPIX
CR 2001-102885 [11]; 2001-137933 [14]; 2002-507280 [54]; 2002-666713 [71]; 2003-246668 [24]; 2003-255218 [25]; 2003-566587 [53]
DNC **C2001-142847**
TI Refolding recombinant **proteins** involves maintaining **protein** at specified **pH** in presence of chaotrophic and reducing agents, and decreasing **pH** of solution gradually to

induce renaturation of the **protein.**
DC B04 D16
IN LIN, X
PA (OKLA-N) OKLAHOMA MEDICAL RES FOUND
CYC 95
PI WO 2001055174 A2 20010802 (200151)* EN 16 C07K001-14 <--
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG UZ VN YU ZA ZW
AU 2001024649 A 20010807 (200174) C07K001-14 <--
US 2001044521 A1 20011122 (200176) C07K001-02 <--
EP 1255769 A2 20021113 (200282) EN C07K001-113 <--
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR
US 6583268 B2 20030624 (200343) C07K014-00
JP 2003523363 W 20030805 (200353) 25 C07K001-14 <--
US 2003199676 A1 20031023 (200370) C07K014-00
ADT WO 2001055174 A2 WO 2000-US35632 20001228; AU 2001024649 A AU 2001-24649 20001228; US 2001044521 A1 Provisional US 2000-177836P 20000125, Provisional US 2000-178368P 20000127, Provisional US 2000-210292P 20000608, Provisional US 2000-210306P 20000608, US 2000-752878 20001228; EP 1255769 A2 EP 2000-988440 20001228, WO 2000-US35632 20001228; US 6583268 B2 Provisional US 2000-177836P 20000125, Provisional US 2000-178368P 20000127, Provisional US 2000-210292P 20000608, Provisional US 2000-210306P 20000608, US 2000-752878 20001228; JP 2003523363 W WO 2000-US35632 20001228, JP 2001-561026 20001228; US 2003199676 A1 Provisional US 2000-177836P 20000125, Provisional US 2000-178368P 20000127, Provisional US 2000-210292P 20000608, Provisional US 2000-210306P 20000608, Cont of US 2000-752878 20001228, US 2003-420044 20030417
FDT AU 2001024649 A Based on WO 2001055174; EP 1255769 A2 Based on WO 2001055174; JP 2003523363 W Based on WO 2001055174; US 2003199676 A1 Cont of US 6583268
PRAI US 2000-210306P 20000608; US 2000-177836P 20000125;
US 2000-178368P 20000127; US 2000-210292P 20000608;
US 2000-752878 20001228; US 2003-420044 20030417
IC ICM **C07K001-02**; **C07K001-113**; **C07K001-14**; C07K014-00
ICS C12P021-06
ICA C12N015-09
AB WO 200155174 A UPAB: 20031030
NOVELTY - Refolding (M) recombinant **proteins** involves maintaining the **protein** at a **pH** of 9.0 or greater, in presence of one or more chaotrophic and reducing agents, and decreasing the **pH** of the solution gradually over about 24 hours to **pH** 8.0 to induce renaturation of a portion of the **protein** , so that it qualitatively exhibits a biological activity and structure characteristic of the **protein.**
USE - (M) is useful for refolding recombinant **proteins** (claimed).
Dwg.0/0
FS CPI
FA AB; DCN
MC CPI: **B04-C01**; B04-N03; **B11-B**; B11-C09; D05-H13; D05-H17
TECH UPTX: 20010910
TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Method: The **pH** is decreased in increments equivalent to 0.2 **pH** units per 24 hours. The **protein** is maintained at a **pH** of greater than 9.0 for a period of at least 24 hours. The **pH** is decreased by

addition of acid, or by dilution or dialysis into a solution of lower **pH.** The chaotrophic and denaturing reagents are selected from between 0.5 and 1.0 M urea, 0.1-100 mM beta-mercaptoethanol, 0.1-100 mM dithiothreitol (DTT), 0.1-10 mM reduced glutathione and 0.1-10 mM oxidized glutathione. The **protein** is **extracted** from bacterial inclusion bodies, preferably **Escherichia coli**, and dissolved at a final **pH** of between greater than 9 and 10, preferably above 10.0, more preferably above 12.0. The **pH** is decreased over a period of at least about 36 hours. The **pH** of the solution is reduced more quickly at the higher **pH** and more gradually nearer the physiological **pH** range of the **protein.** (M) comprises the additional step of **separating protein** species which exhibits biological activity from inactive, or wrongly folded, **protein** species.

ABEX UPTX: 20010910

EXAMPLE - Expression plasmids were transfected into an appropriate host, such as the BL21 (DE3) strain of **Escherichia coli** and plated on ZB/Ampicillin plates, which selected the desired recombinant organisms. A single colony from each construct was inoculated into 100 ml of ZB/ampicillin media and grown. 20 ml of the overnight culture was inoculated into 1 L of LB/ampicillin, and shaken at 37degreesC till OD600 reached 0.4-0.6. IPTG was added, shaken for 3 hours and cells were **centrifuged** and resuspended in 20 ml of TN/1% Triton X-100. 10 mg lysozyme was added, frozen at -20degreesC overnight, thawed, and 20 microl 1 M MgSO4 and 100 microg DNAase was added and stirred until the bacterial DNA was completely dissolved. 250 ml of TN/1% Triton was added, stirred and **centrifuged.** The pellet was dissolved in 10 ml of 8 M urea solution, beta-mercaptoethanol was added and the solution was ultracentrifuged. The OD280 of the solution containing the inclusion bodies was adjusted to 5.0 with the 8 M urea solution. The final solution contained 10 mM beta-mercaptoethanol, 10 mM dithiothreitol (DTT), 1 mM reduced glutathione (GSH), and 0.1 mM oxidized glutathione (GSSG). The above solution was rapidly diluted into 20 volumes of 20 mM TRIS base, the **pH** was adjusted to 9.0, and then slowly adjusted to 8.0 with 1 M HCl, by adjusting **pH** to 8.8 for 24 hours then 8.6 for 24 hours, etc., until the **pH** was 8.0. The refolded material was then concentrated by ultrafiltration, and **separated** by gel filtration. The S-300 fractions were checked by running a non-reduced SDS-PAGE. The wrongly refolded **protein** ran at a very high molecular weight, while folded **proteins** ran at a normal molecular weight. The refolded peak from the S-300 column was further **purified** with a FPLC Resource-Q or Resources-S column, and the enzyme was eluted from the column with a linear gradient of NaCl.

L84 ANSWER 7 OF 11 WPIX COPYRIGHT 2004 THOMSON DERWENT on STN
AN **1999-337255** [28] WPIX
DNC **C1999-099101**
TI **Purifying** a **target protein** from a crude or partially **purified protein** solution.
DC A25 A89 A96 A97 B04
IN GUINN, M R
PA (BAXT-N) BAXTER BIOTECH TECHNOLOGY SARL
CYC 1
PI US 5907035 A 19990525 (199928)* 12 A61K035-14
ADT US 5907035 A Provisional US 1996-18212P 19960523, US 1997-861587 19970522
PRAI US 1996-18212P 19960523; US 1997-861587 19970522
IC ICM A61K035-14
ICS C07K014-00; C07K016-00; C07K017-00
AB US 5907035 A UPAB: 20011211
NOVELTY - **Purifying** a **target protein** from a crude or partially **purified protein** solution comprises the use of aqueous two-phase systems.
DETAILED DESCRIPTION - **Purifying** a **target**

protein comprises:
(a) obtaining a crude solution of the **target protein** having at least one surface active electron-rich amino acid;
(b) adding a salt and a water-soluble hydrophobic molecule to the solution to form a two-phase aqueous system containing a salt phase and a hydrophobic molecule phase, the **target protein** being in the salt phase;
(c) **separating** the salt phase from the hydrophobic molecule phase;
(d) adding a polymer-chelator metal complex charged with a transition metal ion to the salt phase to form a second two-phase aqueous system comprising a polymer-chelator phase containing the **protein**; and
(e) dissociating the polymer-chelator metal complex from the **target protein**, to give the **purified target protein.**
USE - The process is used for **purifying proteins**, especially the recombinant mutant hemoglobin rHb1.1, from crude **protein** solutions.
ADVANTAGE - The process overcomes the problems associated with Cu (II)-catalyzed oxidation of the heme-containing **protein**, by making the ligand highly selective for the **target protein.** The ligand is also selective enough to overcome problems associated with nitrogen-containing compounds inhibiting binding of the **protein** to the ligand. **Protein** solubility is improved and potential precipitation of **proteins** by the salt is prevented.
Dwg.0/0

FS CPI
FA AB; DCN
MC CPI: A12-L04A; A12-W11L; B04-B04D2; B04-N02; B05-A01B; B05-A03A; B05-A03B; B05-C01; B05-C05; B10-A12C; B10-B01B; B10-B02J; **B11-B**

TECH UPTX: 19990719
TECHNOLOGY FOCUS - POLYMERS - Preferred Components: The water-soluble hydrophobic molecule or the polymer is a polyalkylene oxide, preferably polyethylene glycol of molecular weight 400-20000 (especially 1000-8000) (no units given), or polypropylene oxide.

TECHNOLOGY FOCUS - INORGANIC CHEMISTRY - Preferred Components: The salt is sodium, ammonium or magnesium sulfate, or potassium phosphate. The transition metal ion is Fe, Ni, Zn, Co or Cu, preferably Cu.

TECHNOLOGY FOCUS - ORGANIC CHEMISTRY - Preferred Components: The chelator is N,N,N'-tris(carboxymethyl)ethylenediamine, iminodiacetic acid or diethylene triamine. The polymer-chelator metal complex is poly(ethylene)methyl ether-diethyltriamine or iminodiacetic acid-polyethylene glycol (IDA-PEG), preferably Cu(II)IDA-PEG. Preferred Process: The polymer-chelator metal complex is dissociated from the **protein** by lowering the **pH**, adding a competing electron-donor, or adding a strong chelator. The competing electron donor is NH4Cl. The strong chelator is ethylenediamine tetraacetic acid (EDTA). Steps (b) and (c) may be repeated prior to step (d). The crude solution in (a) may be prepared by lysing cells containing the **target protein.**

TECHNOLOGY FOCUS - BIOLOGY - Preferred Components: The **target protein** is hemoglobin, preferably recombinant hemoglobin, especially recombinant mutant hemoglobin, most especially rHb1.1. The **target protein** has more than one surface active, electron-rich amino acid. The amino acid is lysine, arginine, histidine, cysteine, glutamic acid, or aspartic acid.

ABEX UPTX: 19990719
EXAMPLE - **Escherichia coli** was grown and fermented.

The cells were lysed using a Nitro cell disruptor. This gave a crude lysate containing mutant hemoglobin rHb1.1.
Aqueous two-phase systems were prepared by fully dissolving MgSO4.7H2O in the cell lysate above, followed by the addition of stock 50 weight % polyethylene glycol (PEG) 1000 solution. The composition was then equilibrated for 30 minutes at 4 degrees C. Phase **separation** was accomplished by **centrifugation** at 2000 rpm for 5 minutes at 4 degrees C.
The cell debris partitioned in the top phase, forming a viscous, gelatinous top phase. The top phase was carefully removed and the salt-rich phase (containing rHb1.1) was back-**extracted** with PEG 1000 at 4 degrees C. More PEG 1000 was added to form a two-phase system of about the original composition. Cu (II) iminodiacetic acid-polyethylene glycol (Cu (II) IDA-PEG) (9.6 weight %) was added in increasing molar excesses of 24.5, 73.5 and 147.1 and the top phase **extract** was analyzed by SDS-PAGE and ultraviolet-visible absorption.
Increasing the ligand loading resulted in an increase in rHb1.1 partitioning. The ligand was selective for hemoglobin. At the highest loading tested, complete PEG-phase partitioning of hemoglobin was observed as monitored by the disappearance of Soret absorption at 410 nm in the salt-phase.

L84 ANSWER 8 OF 11 WPIX COPYRIGHT 2004 THOMSON DERWENT on STN
AN **1998-396844** [34] WPIX
DNC **C1998-120083**
TI Preparation of recombinant human tumour necrosis alpha-factor - by subjecting **Escherichia coli** cell **extract** to series of **chromatographic** procedures, followed by gel filtration.
DC B04 D16
IN BUMYALIS, V V; DZERSKAITE, I K; KOROBKO, V G
PA (BIOF-R) BIOFA STOCK CO
CYC 1
PI RU 2101292 C1 19980110 (199834)* 5 C07K001-36 <--
ADT RU 2101292 C1 SU 1992-5062556 19920922
PRAI SU 1992-5062556 19920922
IC ICM **C07K001-36**
ICS C12P021-00
AB RU 2101292 C UPAB: 19980826
Recombinant human necrosis tumour alpha -factor (alpha -FNO) is prepared from **Escherichia coli** VKPMV-3967 cells by a series of **chromatographic** procedures carried out on the cell **extract** , followed by gel-filtration.
USE - The method may be useful in biotechnology and microbiological industries and in medicine.
ADVANTAGE - The method gives yields of up to 32% and the product can be used in therapeutics.
Dwg.0/0
FS CPI
FA AB
MC CPI: B04-F10A3; B04-H0600E; D05-H17A2

L84 ANSWER 9 OF 11 WPIX COPYRIGHT 2004 THOMSON DERWENT on STN
AN **1997-214814** [20] WPIX
DNC **C1997-069480**
TI Removal of cells of genus **Escherichia** from fermentation broth - comprises adding **polyethylene imine** to broth before membrane-**separation** of cells.
DC A26 A96 B05 D16 E19
IN NAKAMURA, T; TANABE, T
PA (AJIN) AJINOMOTO CO INC; (AJIN) AJINOMOTO KK
CYC 9
PI EP 768374 A1 19970416 (199720)* EN 9 C12N001-02

R: DE FR GB IT
JP 09164323 A 19970624 (199735) 5 B01D061-14
KR 97020168 A 19970528 (199821) B01D061-14
BR 9605094 A 19980707 (199834) C12N001-02
US 5814513 A 19980929 (199846) C12N001-02
EP 768374 B1 20030129 (200309) EN C12N001-02
R: DE FR GB IT
DE 69625981 E 20030306 (200325) C12N001-02
CN 1155580 A 19970730 (200375) C12N001-20
ADT EP 768374 A1 EP 1996-307416 19961011; JP 09164323 A JP 1996-267016 19961008; KR 97020168 A KR 1996-45322 19961011; BR 9605094 A BR 1996-5094 19961011; US 5814513 A US 1996-732116 19961015; EP 768374 B1 EP 1996-307416 19961011; DE 69625981 E DE 1996-625981 19961011, EP 1996-307416 19961011; CN 1155580 A CN 1996-121049 19961012
FDT DE 69625981 E Based on EP 768374
PRAI JP 1995-265275 19951013
REP DE 3326888; EP 184882; FR 2552674; JP 60078588
IC ICM B01D061-14; C12N001-02; C12N001-20
ICS B01D061-16; C12M001-12; C12P013-04
ICI C12N001-20, C12R001:19
AB EP 768374 A UPAB: 19970516
Cells are removed from a fermentation broth obtd. by culturing a microorganism of the genus **Escherichia**, by: (a) adding **polyethyleneimine** to the broth, and (b) filtering the mixture through a membrane to **separate** the cells.
The fermentation broth **pH** is adjusted from 3 to 7. The membrane used is an ultrafiltration membrane or microfiltration membrane. The amount of **polyethyleneimine** added is 0.0005-0.5 weight% such that the broth is heated at 50-130 deg. C before or after the addition
USE - The process is useful in forming a clear cell-free solution without **centrifugation** by removing cells, cell debris and dissolved high mol.weight impurities.
ADVANTAGE - A high membrane permeation rate is obtd. through pretreatment under mild conditions, avoiding the need for large-sized equipment, and increasing the efficiency of the membrane permeation.
Dwg.0/2
FS CPI
FA AB; DCN
MC CPI: A05-J07; A12-W11L; B04-F01; B04-F10A3; **B11-B**; D05-H13; E10-A20B; E10-B01C

L84 ANSWER 10 OF 11 WPIX COPYRIGHT 2004 THOMSON DERWENT on STN
AN **1989-294240** [41] WPIX
DNC **C1989-130273**
TI Treating insoluble fused heterogeneous **protein** - with acid and **protein** denaturant for solubilisation, then alkali for **purification** and acid for refolding.
DC B04 D16
IN YOSHIKAWA, K
PA (TOYJ) TOSOH CORP
CYC 5
PI EP 336324 A 19891011 (198941)* EN 7
R: DE FR GB
JP 01257491 A 19891013 (198947)
US 5043430 A 19910827 (199137)
EP 336324 B1 19950222 (199512) EN 8 C07K001-113 <--
R: DE FR GB
DE 68921229 E 19950330 (199518) C07K001-113 <--
ADT EP 336324 A EP 1989-105735 19890331; JP 01257491 A JP 1988-85284 19880408; US 5043430 A US 1989-335798 19890410; EP 336324 B1 EP 1989-105735 19890331; DE 68921229 E DE 1989-621229 19890331, EP 1989-105735 19890331
FDT DE 68921229 E Based on EP 336324
PRAI JP 1988-85284 19880408

REP 3.Jnl.Ref; EP 192629; 2.Jnl.Ref
IC C07K003-08; C07K015-12; C12N015-18; C12P021-02
ICM **C07K001-113**
ICS **C07K001-14**; C07K003-08; C07K015-12; C12N015-18; C12P021-02
AB EP 336324 A UPAB: 19930923
The following processes are claimed: (A) a porous for solubilisation treatment of insoluble fused heterogenic **protein** (IFHP) comprising bringing IFHP produced in a host cell into contact with an aqs. acid solution in the presence of a **protein** denaturant; (B) a process for **purification** treatment of IFHP comprising the treatment as in (A) and elevating the **pH** value of the solution by adding an alkali substance; (C) a process for refolding treatment of IFHP comprising the treatment as in (B) then lowering the **pH**, pref. in the presence of a sulphydryl cpd.

Pref. the **protein** denaturant is urea and the alkali substance is monoethanolamine, diethylenetriamine, triethylenetetramine or tetraethylenepentamine.

USE/ADVANTAGE - The processes are suited to rapidly and conveniently treat even such as large quantity of IFHP as has not been treated by previous methods. They can be used to treat e.g. human nerve growth factor (NGF) and a part of human growth hormone (hGH) produced by **E. coli** so that it is refolded and activity is expressed.

0/0
FS CPI
FA AB
MC CPI: B04-B02D4; B04-B04A5; B04-B04J; **B11-B**; D05-C12; D05-H13
ABEQ US 5043430 A UPAB: 19930923
Solubilisation of fusion **proteins** (I) produced by genetically-transformed host organisms comprises contacting (I) with an aq. acid soln. in the presence of a **protein** denaturant (II). An alkaline material (III) is then added to raise the **pH** of the soln. to 10-13 and ppte. impurities from the soln..

(I) comprises, in the N-terminal region, the amino acid sequence of human growth hormone linked via a second **peptide** bond to an amino acid sequence Ile-Gln-Gly-Arg which is linked via a second **peptide** bond to the amino acid sequence of a beta-subunit of human nerve growth factor. (II) is pref. urea. (III) is pref. monoethanolamine, diethylenetriamine, triethylenetetramine or tetraethylenepentamine.

Pref. the **pH** is lowered so that the fusion **protein** undergoes refolding into a biologically-active form.

ABEQ EP 336324 B UPAB: 19950328
A process for solubilising, **purifying** and refolding a fusion **protein** consisting of human nerve growth factor and a part of human growth hormone, comprising the following steps: a) Bringing said fusion **protein** into contact with an aqueous acid solution of **pH** 2 to 5 comprising a denaturing concentraiton of urea, b) adjusting the **pH** to 10 to 13 by adding an alkaline substance and lowering the urea concentration to about 50% of its initial value, thereby precipitating impurities, which are removed, whereas the fusion **protein** consisting of human nerve growth factor and a part of human growth hormon remains in solution, c) lowering the **pH** of the supernatant to **pH** 7 to 9, and d) if necessary, reducing the urea concentration.
Dwg.0/0

L84 ANSWER 11 OF 11 WPIX COPYRIGHT 2004 THOMSON DERWENT on STN
AN **1987-334948** [47] WPIX
DNC **C1987-142952**
TI **Purifying** immunogenic plasmodium **polypeptide** - from recombinant cell cultures by selective precipitation, then ion exchange and reverse phase **chromatography.**
DC B04 D16
IN DEPHILLIPS, P A; FOLENAWASS, G M; SITRIN, R D; ZABRISKIE, D W

PA (DPHI-I) DE PHILLIPS P A; (SMIK) SMITHKLINE BECKMAN CORP
CYC 21
PI WO 8706939 A 19871119 (198747)* EN 40
W: AU DK FI JP NO US
EP 252588 A 19880113 (198802) EN
R: AT BE CH DE ES FR GB GR IT LI LU NL SE
AU 8774400 A 19871201 (198809)
NO 8800080 A 19880321 (198817)
PT 84850 A 19880527 (198826)
DK 8800102 A 19880111 (198830)
ZA 8703338 A 19880621 (198834)
FI 8805237 A 19881111 (198931)
JP 01502556 W 19890907 (198942)
ADT WO 8706939 A WO 1987-701115 19870511; EP 252588 A EP 1987-304133 19870508;
ZA 8703338 A ZA 1987-3338 19870511; JP 01502556 W JP 1987-503190 19870511
PRAI US 1986-861810 19860512
REP 2.Jnl.Ref; A3...8928; EP 191748; EP 192626; EP 22242; No-SR.Pub;
4.Jnl.Ref; GB 2154592; WO 8600911
IC A61K039-01; **C07K001-14**; C07K003-18; C12N007-00; C12P021-02
AB WO 8706939 A UPAB: 19930922
Purification of immunogenic **polypeptides** (I),
comprising at least 4 tandem repeat units of Plasmodium falciparum CS **protein**, from a clarified cell lysate from a recombinant **E .coli** host cell culture, comprises (1) selective precipitation of bacterial contaminants; (2) selective precipitation of (I) from the supernatant;
(3) resolubilising the ppte and selective precipitation of bacterial contaminants;
(4) applying the (I) solution to an ion-exchange support and (5) treating the eluted (I)-containing fraction with solid, hydrophobic support, then eluting adsorbed (I) with a polar organic solvent.
Step (1) is with **polyethylene imine** (PEI); (2) is by salting out; (3) by adjusting to **pH** below 2.5; and (5) is with a 2-18C alkyl-modified support, eluting with 1-3C alcohols, MeCN or THF.
USE/ADVANTAGE - (I) are useful in vaccines against P.falciparum infections (malaria). The **purified** material contains no measurable amts of unwanted **polypeptides** and **proteins**, not over 2ng/mg DNA and less than 10 endotoxin units/mg.
0/0
FS CPI
FA AB
MC CPI: B02-V02; **B04-C01**; **B11-B**; B12-B03; D05-C12

=> d his

(FILE 'HOME' ENTERED AT 13:50:29 ON 04 AUG 2004)
SET COST OFF

FILE 'WPIX' ENTERED AT 13:50:35 ON 04 AUG 2004
L1 1 S US20040049012/PN OR (WO2003-US28007 OR US2002-408653#)/AP,PRN
E R14108+ALL/DCN
L2 437 S E1
L3 4061 S (POLYETHYLENEIMIN? OR POLY()(ETHYLENEIMIN? OR ETHYLENE IMIN?)
L4 4184 S L2,L3
E R06645+ALL/DCN
L5 1561 S E1
L6 52174 S A212/M0,M1,M2,M3,M4,M5,M6 OR L5
E R10140+ALL/DCN
E RA0140+ALL/DCN
E RAO140+ALL/DCN

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                E RA0140/DCN
                E RAO140/DCN
                E RA014O/DCN
L7          64481 S E3-E17 OR A220/M0,M1,M2,M3,M4,M5,M6
                E MAGNESIUM/DCN
                E E3+ALL
L8           1380 S E2
L9          52176 S L6,L8
                E CALCIUM/DCN
                E E86+ALL
L10          2033 S E2
                E CALCIUM/DCN
                E E3+ALL
L11          1308 S E2
L12         64487 S L7,L10,L11
L13          1412 S C07K001-14/IPC
                E D HIS
                E ESCHER/BI,ABEX
L14         15300 S E2-E58
L15             5 S E63-E65,E69
L16             6 S E78,E79
L17         16681 S L13-L16
L18         10777 S ("E" COLI)/BIX
L19         23597 S L17,L18
L20           844 S L19 AND (B11-B OR C11-B)/MC
L21          2074 S L19 AND C07K001/IPC
L22          2577 S L20,L21
L23            20 S L22 AND L9
L24            29 S L22 AND L12
L25            12 S L22 AND L4
L26            37 S L23-L24
L27          5650 S L19 AND (B04-C01? OR C04-C01?)/MC
L28           758 S L22 AND L27
L29             8 S L28 AND L26
L30            37 S L26,L29
L31            10 S L30 AND COLI
L32            23 S L30 AND L13
L33            32 S L31,L32
L34             5 S L30 NOT L33
                  SEL DN AN 5
L35             1 S E1-E2
                  SEL DN AN L33 2
L36             1 S E3-E4
L37           319 S C07K014-245/IPC
L38         22362 S L14,L15,L16,L18,L37
L39            45 S L38 AND C07K004/IPC
L40           575 S L38 AND (B11-B OR C11-B)/MC
L41           620 S L39,L40
L42             6 S L41 AND L4
L43             7 S L41 AND L9
L44             9 S L41 AND L12
L45            19 S L42-L44
L46             4 S L45 AND (B04-C01? OR C04-C01?)/MC
                  SEL DN AN 4 L46
L47             1 S E5-E6
L48            14 S L45 NOT L1,L35,L36,L46,L47
L49            11 S L48 AND PH/BIX
                  SEL DN AN 2 3 11
L50             3 S E7-E12
L51             6 S L1,L35,L36,L47,L50
L52           140 S L41 AND PH/BIX NOT L42-L51
L53           139 S L52 AND COLI/BIX
L54           105 S L53 AND ESCH?/BIX
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L55          56 S L53 AND "E COLI"/BIX
L56         139 S L53 AND L54,L55
L57          76 S L56 AND (PH(2W)(4 OR 5))/BIX
L58          69 S L56 AND (PH(1W)(4 OR 5))/BIX
L59          26 S L56 AND (PH(W)(4 OR 5))/BIX
L60          26 S L59 AND COLI/BIX
L61           0 S L56 AND L2-L12
L62           8 S L56 AND L13
L63           2 S L56 AND C07K001-36/IPC
L64           8 S L62,L63
                SEL DN AN 2 7
L65           2 S E13-E17
L66           8 S L51,L65
L67          26 S L56 AND B04-C01?/MC
L68           2 S L56 AND C04-C01?/MC
L69          27 S L67,L68
                SEL DN AN 5 18
L70           2 S E18-E21
L71          10 S L66,L70
L72          81 S L38 AND L13
L73          34 S L38 AND C07K001-36/IPC
L74         105 S L72,L73
L75          36 S L74 AND (B04-C01? OR C04-C01?)/MC
L76          69 S L74 NOT L75
L77          66 S L76 AND COLI/BIX
                SEL DN AN 28
L78           1 S E22-E23
L79          11 S L71,L78 AND L1-L78
                E GEHANT/AU
L80          11 S E7,L79
L81          11 S L80 AND ("E" COLI OR ESCHER? OR COLI OR PH OR TARGET? OR ?PRO
L82          11 S L81 AND (SEPARAT? OR EXTRACT? OR ISOLAT? OR PURIF?)/BIX
L83           9 S L82 AND (CENTRIF? OR ?CHROMATOG?)/BIX
L84          11 S L82,L83

     FILE 'WPIX' ENTERED AT 14:46:56 ON 04 AUG 2004

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